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(54) Title: A METHOD FOR REGULATING GENES WITH ELECTROMAGNETIC RESPONSE ELEMENTS

(57) Abstract: A non-invasive method for gene regulation during gene therapy comprises the steps of introducing electromagnetic field response elements into a gene promoter not having any electromagnetic field response elements to serve as switches for regulating exogenously introduced genes, and applying an electromagnetic field to the introduced electromagnetic field response elements to induce gene expression. In this way, a safer, more effective, and more precise method for gene therapy is provided of inducing production of desired genetic products.

A METHOD FOR REGULATING GENES WITH ELECTROMAGNETIC
RESPONSE ELEMENTS

BACKGROUND OF THE INVENTION

5 Throughout this application, various publications are referenced to by arabic numerals within parentheses. Disclosures of these publications in their entireties are hereby incorporated by reference into this application to more fully describe the state of the art to which this invention
10 pertains. Full bibliographic citations for these references may be found at the end of this application, preceding the claims.

Gene therapy was proposed approximately 20 years ago as a way to ameliorate genetic defects by providing a source for
15 missing essential genetic components. The injection of copies of the gene responsible for the production of a specific protein directly into the targeted area by means of a viral vector was considered a mode of insuring that the protein required would be synthesized at the site where it was needed.
20 This approach offered a distinct advantage over prior conventional treatment of metabolic diseases, which required continuous injection of gene product from exogenous sources.

The principle behind gene therapy is simple; however, practical application has been difficult. Failure of early
25 gene therapy was mainly due to three problems. Firstly, there were difficulties in efficiently transducing primary quiescent human cells *in vivo*. Secondly, there were strong immune

responses to the gene therapy vectors, as well as to the foreign therapeutic transgenes that rapidly eliminated transgene expressing cells in humans. Thirdly, there was an ability of many cell types to shut off the viral promoters
5 that controlled transgene expression in humans.

One positive outcome of these early efforts of gene therapy was the demonstration that introducing cloned genes into humans could be safe, with little or no morbidity. More recently, new vectors have been engineered, including
10 adenoviruses and even naked DNA, enhancing the efficiency of *in vivo* gene delivery and reducing the immunogenicity of vectors and transgenes.

There is a need for a safer, more effective, and more precise method of gene therapy.

SUMMARY OF THE INVENTION

The present invention provides a unique method for gene regulation, using electromagnetic response elements. In the present invention, exogenously introduced genes, in gene therapy, are regulated by the introduction of electromagnetic response elements (EMREs) into the gene promoters that do not have them to serve as "switches." Exposure to electromagnetic fields of $8\mu\text{T}$ 60Hz for 30 minutes induces gene expression, because the switches make the gene now responsive to EM fields. The electromagnetic field response elements, therefore, are the "switches." The present invention therefore provides a non-invasive technique in gene therapy.

In this way, a safer, more effective, and more precise method for gene therapy is provided for inducing production of desired gene products. The present invention is therefore an improvement over the invasive character of current gene therapy protocols.

The electromagnetic field response elements, therefore, can be introduced into any gene promoter not having them. Examples are insulin and the cystic fibrosis gene. The electromagnetic field response elements can be introduced into any gene that would supply a missing gene product that the person does not already have due to some genetic consequence.

The present invention not only regulates and programs gene promoters to induce genetic information, but it does so in a patient-friendly manner.

Applying an electromagnetic field to the introduced gene containing the new electromagnetic field response elements induces gene expression.

5 In summary, the present invention in one embodiment provides a non-invasive method for gene regulation during gene therapy, comprising the steps of: introducing electromagnetic field response elements into a gene promoter not having any electromagnetic field response elements to serve as switches for regulating exogenously introduced genes; and applying an
10 electromagnetic field to the introduced electromagnetic field response elements to induce gene expression.

The present invention in another embodiment provides a non-invasive method for gene regulation during gene therapy, comprising the steps of: introducing at least one
15 electromagnetic field response element into a gene promoter not having any electromagnetic field response elements to serve as switches for regulating exogenously introduced genes; and applying an electromagnetic field to each introduced electromagnetic field response element to induce gene
20 expression.

BRIEF DESCRIPTION OF THE FIGURES

Fig. 1 illustrates construction of EMRE-expression vectors; and

5 Fig. 2 illustrates CAT and Luciferase activities, wherein samples in lane 1 were sham-exposed (30 mins), and samples in lanes 2, 3, and 4 were exposed to 8 μ T 60Hz EM fields (30 mins).

DETAILED DESCRIPTION OF THE INVENTION

The present invention in one embodiment provides a non-invasive method for gene regulation during gene therapy, comprising the steps of: introducing electromagnetic field response elements into a gene promoter not having any electromagnetic field response elements to serve as switches for regulating exogenously introduced genes; and applying an electromagnetic field to the introduced electromagnetic field response elements to induce gene expression.

- 10 The introduced electromagnetic field response elements may be nCTCTn sequences in an HSP70 gene promoter. A number of the nCTCTn sequences may be 3. The nCTCTn sequences may lie between about -230 and about -160 in the HSP70 gene promoter.

The introduced electromagnetic field response elements may be nCTCTn sequences in a c-myc gene promoter. A number of the nCTCTn sequences may be 8. The nCTCTn sequences may lie between about -1257 and about -353 in the c-myc gene promoter.

The electromagnetic field is preferably applied at a field strength of about 8 μ T and a frequency of about 60Hz for a time of about 30 minutes.

The present invention in another embodiment provides a non-invasive method for gene regulation during gene therapy, comprising the steps of: introducing at least one electromagnetic field response element into a gene promoter not having any electromagnetic field response elements to

serve as switches for regulating exogenously introduced genes; and applying an electromagnetic field to each introduced electromagnetic field response element to induce gene expression.

- 5 Each introduced electromagnetic field response element may be an nCTCTn sequence in an HSP70 gene promoter. Each introduced electromagnetic field response element may be an nCTCTn sequence in a c-myc gene promoter. The electromagnetic field is preferably applied at a field strength of about 8 μ T and a
10 frequency of about 60Hz for a time of about 30 minutes.

Low frequency electromagnetic (EM) fields induce increased expression of the stress response gene HSP70 (6) (2). There are several parallels in the biochemical pathways induced by electromagnetic fields and heat shock, but there are striking
15 differences as well. Both pathways involve the binding of heat shock factor 1 (HSF1) to a heat shock element (HSE), but regulation of HSP70 gene expression by electromagnetic fields involves three nCTCTn binding sites in the HSP70 promoter that lie between -230 and -160, upstream from the transcription
20 initiation site. These three nCTCTn sequences appear to act as electromagnetic field response elements (EMREs), since the ability of an electromagnetic field to induce stress proteins gradually disappears as the EMREs are mutated one by one (7) (9). Removal of EMREs by mutation does not affect the
25 response to heat shock, since the heat shock domain is downstream from the electromagnetic field domain in the HSP70 promoter, i.e., between -106 and -67 (6) (8) (9).

A 900bp region in the *c-myc* promoter (-1257 to -353) is responsive to electromagnetic fields (5). Recent reanalysis of this 900bp region revealed eight nCTCTn sequences within this DNA fragment. These eight EMREs in the *c-myc* promoter
5 could account for the electromagnetic field sensitivity of the *c-myc* gene, and the resultant increased *c-myc* transcript levels in cells exposed to electromagnetic fields (4).

To determine whether EMREs can serve as switches to regulate
10 exogenously introduced genes, the 900bp fragment of the *c-myc* promoter was placed upstream of CAT (chloramphenicol transferase) or luciferase reporter constructs that were otherwise unresponsive to electromagnetic fields. EMRE-reporter constructs were transfected into HeLa cells and
15 transfectants exposed to electromagnetic fields. Protein extracted from EM field-exposed transfectants showed increased CAT and luciferase activities, whereas no increase in CAT or luciferase was measurable in the unexposed controls. Three
20 kinds of controls were used: transfectants that were sham-exposed, transfectants lacking EMREs, and non-specific protein. These data support the theory that EMREs can be inserted into the promoters of exogenously introduced genes to serve as switches that respond to electromagnetic fields. This
25 would provide a new and powerful non-invasive technique for regulating gene expression during gene therapy.

MATERIALS AND METHODS

Cell culture and transfections

As previously described, HeLa cells were used for transient transfections and the lipofectin method (Gibco/BRL, Cat #
5 18292-011) was used for transfection as described (6) (7) (8).

900bp segment from the c-myc promoter

The 900bp region of the c-myc promoter containing eight copies of nCTCTn extends from -353 (PVUII site) to -1257 (ClaI site).

10

pAH-11-CAT HSP70 deletion construct

A diagrammatic representation of this construct is presented in reference (9). This construct contains the first 111 base pairs upstream from the transcription initiation site and
15 includes the heat shock domain (-106 to -67). There are no nCTCTn binding sites in this construct and it is not responsive to electromagnetic fields (9).

Construction of EMRE-CAT expression vector

Fig. 1A illustrates construction of the EMRE-CAT expression
20 vector (pΔ11+ 900bp + CAT). Plasmid pΔ11-CAT was digested with Hind III and PVU II, harvested from gel. Two oligonucleotides were used for PCR which allowed us to create two enzyme sites and amplify the 900bp region from c-myc promoter.

- 25 1. CCTGAGCTCTTCTTTGATCAGAATCGATA
2. TCTAAGCTTCTTTGATCAGAATCGATG

1 μ l of plasmid (digested with Hinde III and PVUII) was mixed with 3 μ l PCR product, placed at 12°C overnight for ligation and transformed using DH52 bacteria. Clone hybridization verified insert.

5 Construction of EMRE- luciferase expression vector

Fig. 1B illustrates construction of the EMRE- luciferase expression vector (PGL₃ + 900bp + luciferase). A luciferase expression vector (PGL3 Promega) was digested with SacI and SmaI and harvested from a gel. Two oligonucleotides (see
10 above) were used for PCR, which allowed creation of two enzyme sites and amplified the 900bp region from c-myc promoter. 1 μ l of digested plasmid was mixed with 3 μ l PCR product, placed at 12°C overnight for ligation, and transformed using DH52 bacteria. Clone hybridization verified
15 insert.

Protein

Protein was extracted and concentrations determined as previously described (6) (7) (8) (9).

CAT assay

20 CAT assays were performed as previously described (6) (7). Results were quantified using a PhosphorImager and ImageQuant software.

Luciferase assay

Luciferase activity was determined (Luciferase Assay Kit)

(Promega #E1501) and results quantified as suggested by Promega.

Magnetic field exposures of transfectants

Transfectants were exposed and sham-exposed as previously
5 described (7) (9).

Heat shock

Samples from cells that had been heat shocked (43°C) served as positive controls for CAT assay. Petri dishes containing transfectants were wrapped in Parafilm, placed in a mu metal
10 box (to shield them from exposure to the magnetic fields generated by the water bath heating motor) and immersed in the water bath at 43°C for 30 minutes. Petri dishes were removed from the water bath and, following an additional 30 minutes at 37°C, protein was extracted (3) (6).

15 Electromagnetic field exposure system

Two fully functional exposure units provided simultaneous sham and experimental exposures. Exposures used Helmholtz coils (Electric Research and Management, Pittsburgh, PA) that consisted of 19-gauge wire bundles wound 164 times around a
20 square form 13cm long and 14cm wide with 8cm spacing. The coils were energized by a function generator (11 MHz Wavetek Stabilized Function Generator, model 21). A digital multimeter was used to measure the field intensity and verify the systems operation (Fluke 87 digital multimeter). Field
25 parameters were monitored with a Hitachi V-1065 100MHz oscilloscope and calibrated inductive search coil (25X;

Electro-Biology Inc., Parsippany, NJ). Detailed description of the exposure system, including background magnetic fields in the incubator, harmonic distortion, DC magnetic fields and mean static magnetic fields in the incubator, both vertical and horizontal components, can be found in reference (4). Cells were placed on a Plexiglas stand in a horizontal orientation; i.e., the entire area of the dish was exposed to the field. The bottom of the dish was 2cm below the axis level. The height from dish bottom to top surface of liquid was approximately 1.1 cm. The height of the liquid was 0.6cm. The calculated electric field was $\sim 11 \mu\text{V/m}$ for an $8\mu\text{T}$ exposure.

Mu metal shielding

Helmholtz coils were enclosed within Mu metal containers to minimize stray fields during electromagnetic field exposures. Both active (experimental) and sham-exposed coils (controls) were enclosed in a 30 cm high, 15 cm diameter cylindrical mu metal container (.040" thickness) (Amuneal Corp. Philadelphia, PA). The 60Hz shielding factor is (Min.) 90.1 (39.08dB). Sham-exposed controls and experimental exposures are performed simultaneously in identical mu metal containers.

Statistical analyses

A sufficient number of experiments were performed to assure statistical significance. Statistical significance is determined by a multifactor analysis of variance program (INSTAT).

Results

As will be discussed below, it was shown that EMREs increase luciferase activity in transfectants exposed to EM fields.

To determine whether the nCTCTn sequences (EMREs) that are EM field responsive would confer EM field responsiveness to a reporter construct lacking these sequences, a 900bp region from the c-myc promoter containing eight copies of nCTCTn was ligated to a PGL3 plasmid containing a portion of the SV40 promoter and carrying the luciferase gene (see Fig. 1A). This plasmid construct was transfected into HeLa cells and the transfectants exposed to 8 μ T 60Hz fields for 30 minutes, followed by an additional 30 minutes out of the field prior to protein extraction for the luciferase assay. Luciferase activity increased an average of 61%.

Fig. 2A is a bar graph showing luciferase activities. Fig. 2A illustrates: (1) luciferase activity in protein extracted from transfectants containing luciferase construct plus the 900bp insert (sham-exposed); (2) luciferase activity using non-specific protein (negative control) (EM field-exposed); (3) luciferase activity in protein extracted from transfectants containing luciferase construct minus the 900bp insert (EM field-exposed); and (4) luciferase activity in protein extracted from transfectants containing luciferase construct plus the 900bp insert (EM field-exposed).

Therefore, three sets of controls were used: (1) sham-exposed

transfectants that served as controls for electromagnetic field exposure, and showed no significant luciferase activity; (2) transfectants containing the luciferase reporter construct without the 900bp insert served as controls for background and showed no measurable luciferase activity; and (3) non-specific protein served as negative controls with no measurable activity. These transfectants were not responsive to heat shock, as expected from the absence of heat shock consensus sequences (nGAAn) in this plasmid construct.

10 It was also shown that EMREs increase CAT activity in constructs exposed to EM fields. In similar experiments with a CAT reporter construct, the 900bp region from the c-myc promoter containing eight nCTCTn was ligated to pΔ11- CAT (see Fig. 1B), transfected into HeLa cells, and the
15 transfectants exposed to an 8μT 60Hz field for 30 minutes, followed by an additional 30 minutes out of the field prior to protein extraction for the CAT assay. There was an average 60% increase in CAT activity. The same three sets of controls described above were employed in these experiments (see Fig.
20 2B): (1) sham-exposed transfectants served as controls for electromagnetic field exposure, and showed no significant CAT activity; (2) transfectants containing the CAT reporter construct without the 900bp insert (pΔ11-CAT) served as controls for background; protein extracts from these
25 transfectants showed no measurable CAT activity; and (3) non-specific protein served as negative controls. Transfectants with and without the 900bp insert were heat shocked for 30 minutes at 43°C followed by protein extraction after an

additional 30 minutes out of the heat. There was an average 45% increase in CAT activity in heat shocked transfectants. The pΔ11 plasmid contains the heat shock domain, -106 to -67 and therefore response to heat shock served as an additional
5 control.

Therefore, Fig. 2B illustrates: (1) CAT activity in protein from transfectants containing CAT construct plus the 900bp insert (sham-exposed); (2) CAT activity using non-specific protein (negative control) (EM field-exposed); (3) CAT
10 activity in protein from transfectants containing CAT construct minus the 900bp insert (EM field-exposed); and (4) CAT activity in protein from transfectants containing CAT construct plus the 900bp insert (EM field-exposed).

Discussion

15 Because electromagnetic fields penetrate tissues without attenuation, they must penetrate to the cell nucleus with its DNA and interact with moving charges there (1). There are conducting electrons in DNA (12), and direct measurements of electrical transport through DNA have been made. The dynamics
20 of DNA-mediated electron transfer at the femtosecond level have been measured (14). Conduction in DNA appears to depend on specific structure, since different DNA sequences have different conductivities (10). Therefore, electromagnetic fields could theoretically interact preferentially with
25 specific DNA sequences, and the nCTCTn sequences (EMRES) in the HSP70 and c-myc promoters used in these studies may be such sequences.

We have shown that these sequences are critical for electromagnetic field responsiveness in our experiments, and other data appear to support this. In totally unrelated investigations, one study showed that low frequency
5 electromagnetic field stimulation in nigro-striatal lesioned rats with chromaffin transplants induced changes in the subventricular zones and led to significant motor improvements in a rat Parkinson model (13). A second report from the same laboratory has used differential display to analyze possible
10 alterations in DNA of electromagnetic field-exposed chromaffin cells.

Differential bands observed in the EM field-exposed group show changes in gene expression induced by electromagnetic fields. One specific differential band in the EM field-exposed
15 samples, containing 349bp, was sequenced. In an independent analysis of this DNA fragment, we have identified three copies of the electromagnetic field response element (nCTCTn) that we described herein. A computer search may determine whether this 349bp DNA fragment is contained in the promoters of any
20 known genes, possibly a specific gene related to the differentiation process of chromaffin cells.

Electromagnetic fields induce gene expression (2) (9) and activation of the gene by electromagnetic fields requires
25 specific EMREs, which control genes when placed upstream of reporter constructs. Their ability to confer electromagnetic field responsiveness suggests the use of EMREs in the control and regulation of gene therapy. The characterization of a

cellular promoter system that can be regulated, such as described here, provides a novel, noninvasive, technique for the regulation of transgene expression in humans without interfering with normal physiologic function. The applied
5 electromagnetic field can be directed to the region where the gene product is needed, and, since the electromagnetic field intensities needed to affect EMREs are well below the human perception threshold, their introduction and presence would not be felt by the patient.

10 An example of such application would be the introduction of an exogenous insulin gene containing one or more EMREs placed upstream of the gene. Regulation would be provided by the simple and safe application of electromagnetic fields. The whole operation would be made automatic by having the EM field
15 generating circuit activated by an implanted glucose sensor responsive to pre-set blood glucose levels.

Our results show that the eight nCTCTn sequences (EMREs) in the 900bp DNA fragment from the c-myc promoter are effective in regulating CAT or luciferase activity. However, not all
20 eight EMREs may be needed for a response (9). The EM-induced expression of HSP70 is mediated through three EMREs in the human HSP70 promoter. Electromagnetic field exposure of HSP70 promoter constructs, linked to a CAT reporter gene and containing all three sites, showed more than a three fold
25 increase in CAT activity. Yet, the presence of even one site was sufficient for a 1.5 fold increased CAT response. These data show that even a single EMRE can promote interaction with

electromagnetic fields. The data also suggest that the level of interaction appears to be roughly proportional to the number of EMREs.

According to an embodiment of this invention, then, nCTCTn
5 sequences, taken from the myc promoter, were attached to HSP70 constructs that didn't contain them. The HSP70 promoter has three nCTCTn sequences in the electromagnetic field domain (230-160), but none in the heat shock domain (111-67). When the nCTCTn sequences are inserted into the heat shock domain
10 (which was previously responsive only to heat and not to electromagnetic fields) this promoter construct that previously did not respond to EM fields, now does respond and induces gene expression.

In this way, electromagnetic field response elements, i.e.,
15 nCTCTn sequences from the c-myc promoter, are actively incorporated into the HSP70 promoter and regulate and program gene expression; thus, inserting these nCTCTn sequences into a reporter construct (CAT or Luciferase) that was previously unresponsive to EM fields, renders the gene electromagnetic
20 field-responsive, and induces the gene activity.

Summary

A 900 base pair segment of the c-myc promoter, containing eight nCTCTn sequences, induces c-myc expression by
25 electromagnetic fields. Similarly, a 70bp region of the HSP70 promoter, containing three nCTCTn sequences, induces HSP70 expression by electromagnetic fields. Removal of the

900 base pair segment of the *c-myc* promoter eliminates the ability of electromagnetic fields to induce *c-myc* expression. Similarly, removal of the 70bp region of the HSP70 promoter, with its three nCTCTn sequences, eliminates the response to electromagnetic fields. The nCTCTn sequences apparently act as electromagnetic field response elements (EMREs). To test whether introducing EMREs imparts the ability to respond to applied electromagnetic fields, the 900bp segment of the *c-myc* promoter (containing eight EMREs) was placed upstream of CAT or luciferase reporter constructs that were otherwise unresponsive to electromagnetic fields. EMREs-reporter constructs were transfected into HeLa cells and exposed to 8 μ T 60Hz fields. Protein extracts from EM field-exposed transfectants had significant increases in activity of both CAT and luciferase, compared with identical transfectants that were sham-exposed. Transfectants with CAT or luciferase constructs lacking EMREs remained unresponsive to EM fields; that is, there was no increase in either CAT or luciferase activity. These data support the idea that EMREs can be used as switches to regulate exogenously introduced genes in gene therapy.

Although embodiments of the invention have been described herein, numerous variations and modifications will occur to those skilled in the art without departing from the scope of the invention. The invention is not limited to the embodiments disclosed, and is defined only by way of the following claims.

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What is claimed is:

1. A non-invasive method for gene regulation during gene therapy, comprising the steps of:

introducing electromagnetic field response elements into
5 a gene promoter not having any electromagnetic field response elements to serve as switches for regulating exogenously introduced genes; and

applying an electromagnetic field to the introduced electromagnetic field response elements to induce gene
10 expression.

2. The method as set forth in claim 1, wherein the introduced electromagnetic field response elements are nCTCTn sequences in an HSP70 gene promoter.

3. The method as set forth in claim 2, wherein a number
15 of the nCTCTn sequences is 3.

4. The method as set forth in claim 3, wherein the nCTCTn sequences lie between about -230 and about -160 in the HSP70 gene promoter.

5. The method as set forth in claim 1, wherein the
20 introduced electromagnetic field response elements are nCTCTn sequences in a c-myc gene promoter.

6. The method as set forth in claim 5, wherein a number of the nCTCTn sequences is 8.

7. The method as set forth in claim 6, wherein the nCTCTn sequences lie between about -1257 and about -353 in the c-myc gene promoter.

8. The method as set forth in claim 1, wherein the
5 electromagnetic field is applied at a field strength of about 8 μ T and a frequency of about 60Hz for a time of about 30 minutes.

9. A non-invasive method for gene regulation during gene therapy, comprising the steps of:

10 introducing at least one electromagnetic field response element into a gene promoter not having any electromagnetic field response elements to serve as switches for regulating exogenously introduced genes; and

applying an electromagnetic field to each introduced
15 electromagnetic field response element to induce gene expression.

10. The method as set forth in claim 9, wherein each introduced electromagnetic field response element is an nCTCTn sequence in an HSP70 gene promoter.

20 11. The method as set forth in claim 9, wherein each introduced electromagnetic field response element is an nCTCTn sequence in a c-myc gene promoter.

12. The method as set forth in claim 9, wherein the electromagnetic field is applied at a field strength of about

8 μ T and a frequency of about 60Hz for a time of about 30 minutes.

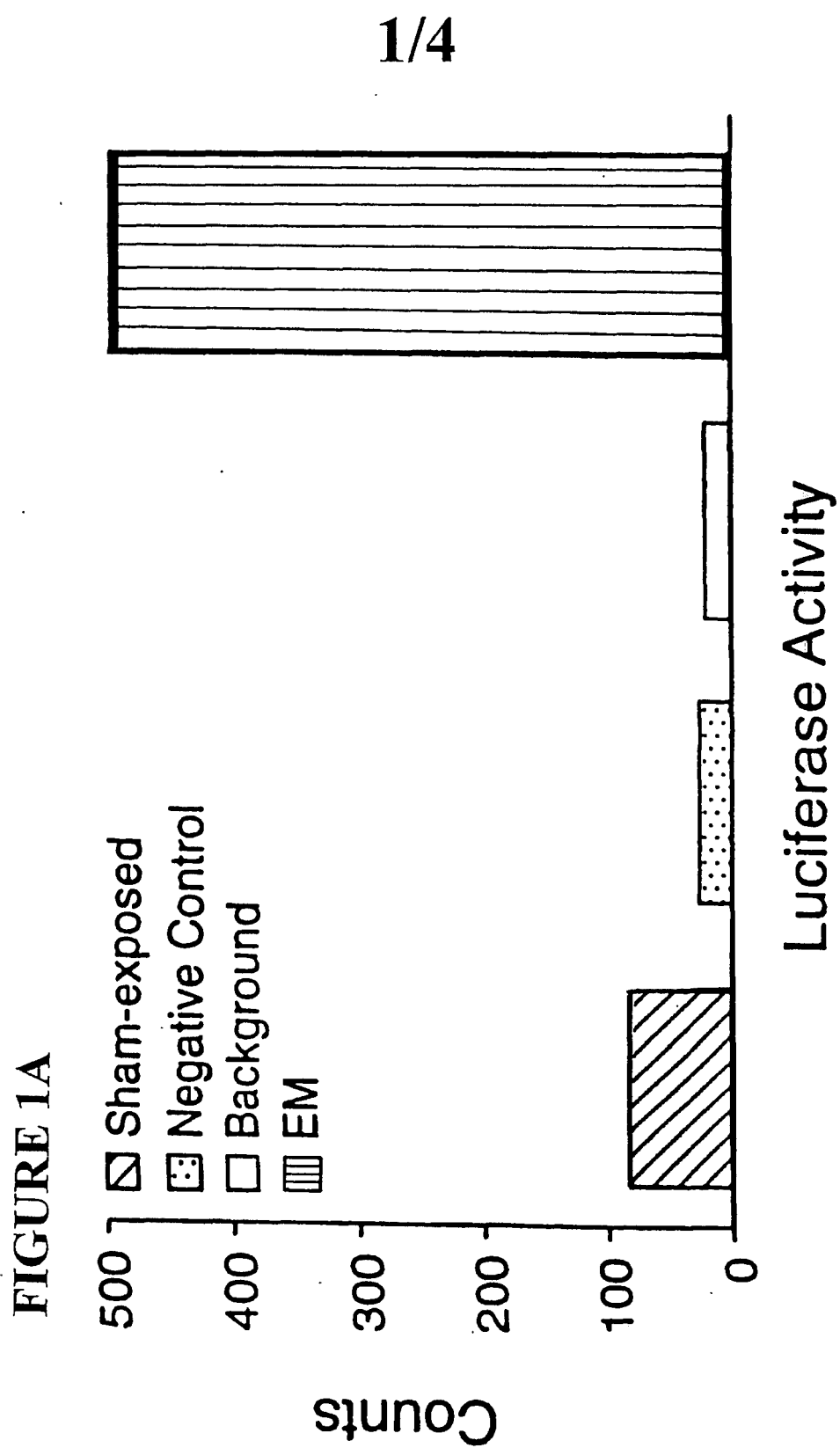
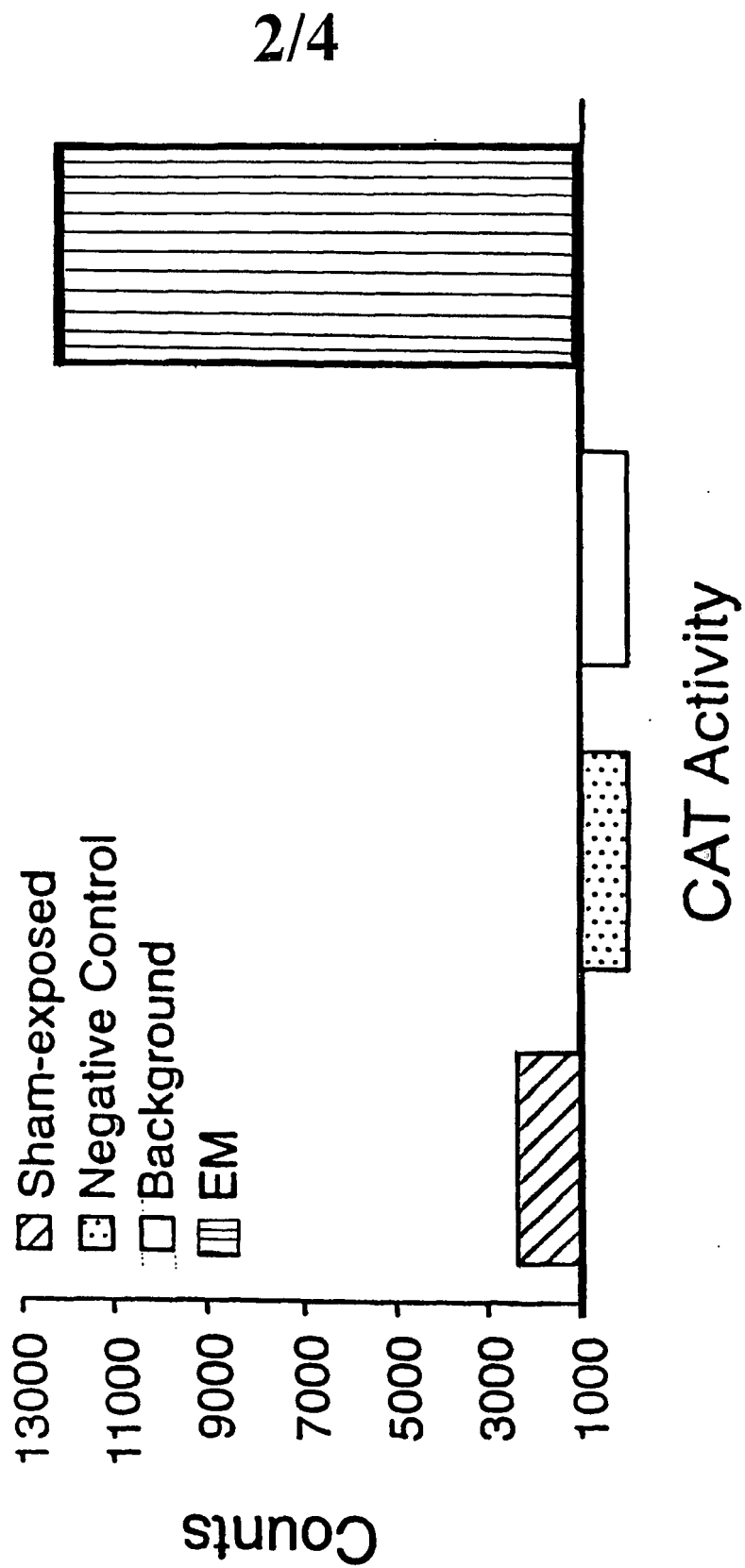
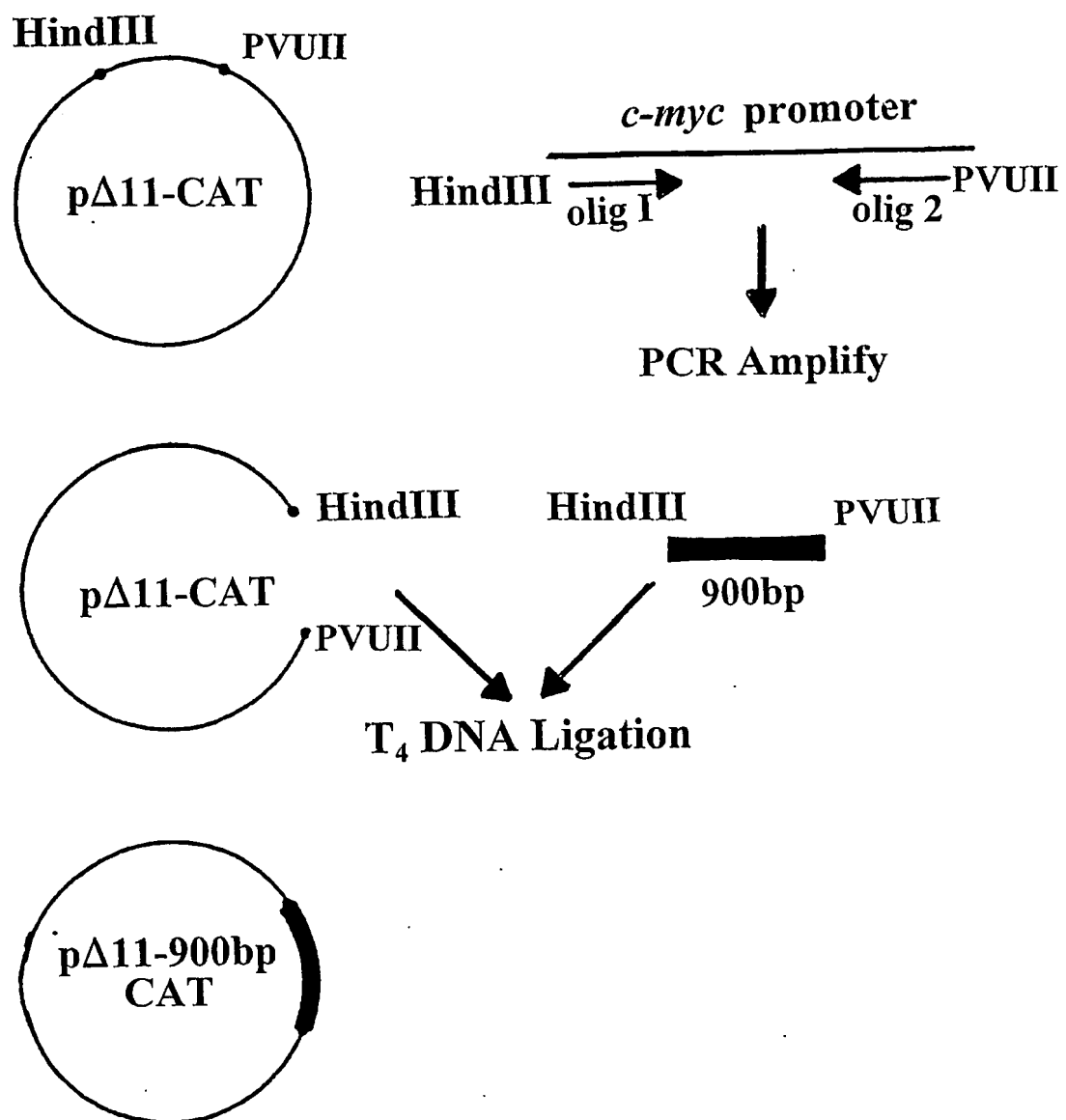


FIGURE 1B



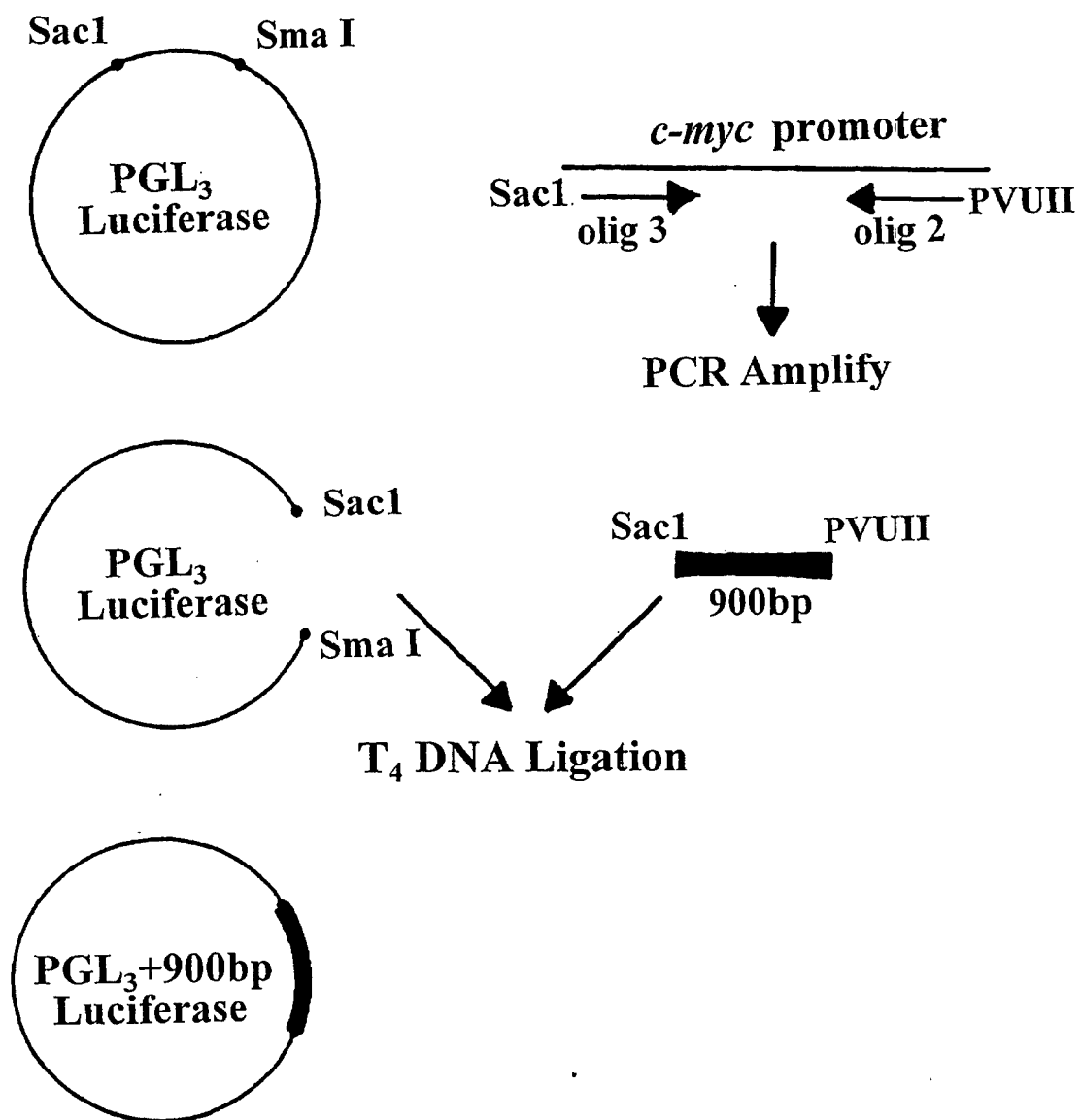
3/4

FIGURE 2A



4/4

FIGURE 2B



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US02/03778

A. CLASSIFICATION OF SUBJECT MATTER												
IPC(7) : C07H 21/04; A01N 43/04; A61K 31/70												
US CL : 536/24.1; 514/44												
According to International Patent Classification (IPC) or to both national classification and IPC												
B. FIELDS SEARCHED												
Minimum documentation searched (classification system followed by classification symbols) U.S. : 536/24.1, 23.1, 23.2, 24.2; 514/44												
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched												
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) Please See Continuation Sheet												
C. DOCUMENTS CONSIDERED TO BE RELEVANT												
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.										
X,P	LIN et al. Regulating genes with electromagnetic response elements. Journal of Cellular Biochemistry. December 2000, Vol. 81, pages 143-148. Entire document.	1-12										
Y	BLANK, M. et al. Electromagnetic fields may act directly on DNA. Journal of Cellular Biochemistry. 1999, Vol. 75, pages 369-374. Entire document.	1-4, 8-10, 12										
Y	LIN et al. Electromagnetic field exposure induces rapid, transitory heat shock factor activation in human cells. Journal of Cellular Biochemistry. 1997, Vol. 66, pages 482-488. Entire document.	1-4, 8-10, 12										
Y	GOODMAN, R. et al. Magnetic field stress induces expression of hsp70. Cell Stress Chaperones. 1998, Vol. 3, No. 2, pages 79-88. Entire document.	1-4, 8-10, 12										
X	LIN et al. Specific region of the c-myc promoter is responsive to electric and magnetic fields. Journal of Cellular Biochemistry. 1994, Vol. 54, pages 281-288. Entire document.	1, 5-8, 9, 11, 12										
Y	JIN et al. Biological and technical variables in myc expression in HL60 cells to 60 Hz electromagnetic fields. Bioelectrochem. Bioenerg. 1997, Vol. 44, No. 1, pages 111-120. Entire document.	1, 5-8, 9, 11, 12										
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.												
* Special categories of cited documents: <table border="0"> <tr> <td>"A" document defining the general state of the art which is not considered to be of particular relevance</td> <td>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</td> </tr> <tr> <td>"E" earlier application or patent published on or after the international filing date</td> <td>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</td> </tr> <tr> <td>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</td> <td>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</td> </tr> <tr> <td>"O" document referring to an oral disclosure, use, exhibition or other means</td> <td>"&" document member of the same patent family</td> </tr> <tr> <td>"P" document published prior to the international filing date but later than the priority date claimed</td> <td></td> </tr> </table>			"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention	"E" earlier application or patent published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone	"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art	"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family	"P" document published prior to the international filing date but later than the priority date claimed	
"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention											
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"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family											
"P" document published prior to the international filing date but later than the priority date claimed												
Date of the actual completion of the international search 14 May 2002 (14.05.2002)		Date of mailing of the international search report 11 JUN 2002										
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703)305-3230		Authorized officer Dr. Henry Yucel Telephone No. (703) 308-1096										

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US02/03778

C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HAN et al. Application of magnetic field-induced heat shock protein 70 for presurgical cytoprotection. Journal of Cellular Biochemistry. 1998, Vol. 71, pages 577-583. Entire document.	1-4, 8-10, 12

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US02/03778

Continuation of B. FIELDS SEARCHED Item 3:

Cancerlit, Medline, Caplus, Biosis, Embase

Search Terms: Electromagnetic control elements, gene therapy, hsp70, c-myc,